इंटरनेट मानक

Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

"जानने का अधिकार, जीने का अधिकार"
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

IS 4446-1 (2008): Chlorophyll, Part 1: Magnesium Complex
[FAD 8: Food Additives]

"ज्ञान से एक नये भारत का निर्माण"
Satyanarayan Gangaram Pitroda
"Invent a New India Using Knowledge"

Act Number 22 of 2005
The Indian Parliament
RIGHT TO INFORMATION

Made Available By
Public.Resource.Org

"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

BLANK PAGE

BRUTUM FULMEN
IGNORANTIA JURIS NON EXCUSAT
PROTECTED BY COPYRIGHT

भारतीय मानक

क्लोरोफिल, खाद्य ग्रेड — विशिष्टि

भाग 1 मैगनीशियम संकुल

(दूसरा पुनरीक्षण)

Indian Standard

CHLOROPHYLL, FOOD GRADE — SPECIFICATION

PART 1 MAGNESIUM COMPLEX

(Second Revision)

ICS 67.220.20

© BIS 2008

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

June 2008

Price Group 2

AMENDMENT NO. 1 APRIL 2011
TO
IS 4446 (PART 1) : 2008 CHLOROPHYLL, FOOD GRADE — SPECIFICATION

PART 1 MAGNESIUM COMPLEX

(Second Revision)

[*Page* 1, *Table* 1, *Sl No.* (ii), *col* 2] — Substitute 'Residual solvents (Acetone, methanol, ethanol, propan-2-ol, hexane), mg/kg, *Max*' *for* 'Residual solvents, mg/kg, *Max* Acetone, methanol, ethanol, propan-2-ol, hexane1)' and delete footnote.

[*Page* 1, *Table* 1, *Sl No.* (ii), *col* 4] — Substitute 'B' *for* '—'.

[*Page* 1, *Table* 1, *Sl No.* (iii), *col* 2] — Substitute 'Dichloromethane, mg/kg, *Max*' *for* 'Dichloromethane 1)' and delete footnote.

[*Page* 1, *Table* 1, *Sl No.* (iii), *col* 4] — Substitute 'B' *for* '—'.

[*Page* 2, *clause* 5.3(d)] — Substitute 'Net quantity' *for* 'Net content'.

(*Page* 3, *Annex* A) — Insert the following new Annex after Annex A:

ANNEX B
[*Table* 1, *Sl No.* (ii) *and* (iii)]

DETERMINATION OF RESIDUAL SOLVENTS AND DICHLOROMETHANE USING HEAD SPACE CHROMATOGRAPHY

B-1 METHOD 1 (DETERMINATION CARRIED OUT IN WATER)

B-1.1 Reagents

B-1.1.1 *Blank*, sample with very low solvent content.

B-1.1.2 *Internal Standard*, 3-methyl-2-pentanone.

B-1.1.3 *Methanol*

Price Group 2

B-1.1.4 *Demineralized Water*

B-1.1.5 *Internal Standard Solution* — Add 50.0 ml water to a 50 ml injection vial and seal. Accurately weigh and inject 15 µl 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.

B-1.1.6 *Blank Solution* — Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of water and 1.0 ml of the internal standard solution. Heat at 60°C for 10 min and shake vigorously for 10 sec.

B-1.1.7 *Samples* — Weigh accurately 0.20 g sample into an injection vial. Add 5.0 ml water and add 1.0 ml of the internal standard solution. Heat at 60°C for 10 min and shake vigorously for 10 sec.

B-1.1.8 *Calibration Solution* — Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of the water and 1.0 ml of the internal standard solution. Weigh the vial accurately to within 0.01 mg. Inject a known volume of the component of interest through the septum and again reweigh the vial. Heat at 60°C for 10 min and shake vigorously for 10 sec.

B-1.2 Procedure

Place the sample, blank and calibration samples in the sample tray of the head-space gas chromatograph — FID system. Analyze using the following analytical conditions.

B-1.2.1 *Column* — Fused silica, length 0.8 m, i.d. 0.53 mm, coated with DB-wax, film thickness 1 µm.

B-1.2.2 *Coupled with*, fused silica, length 30 m, i.d. 0.53 mm, coated with DB-1, film thickness 5 µm.

B-1.2.3

Conditions:

Carrier gas:	Helium
Flow rate:	208 kPa, 5 ml/min
Detector:	FID
Temperatures:	
Injector:	140°C
Oven conditions:	35°C for 5 min, then 5°C/min to 90°C, then 6 min at 90°
Detector:	300°C
Head space sampler	
Sample heating temperature:	60°C
Sample heating period:	10 min
Syringe temperature:	70°C
Transfer temperature:	80°C
Sample gas injection:	1.0 ml in split mode

B-1.3 Calculation

$A \times B \times C/50$ = mg component per injection vial

where

A = relative peak area of the component concerned,
B = mg internal standard, and
C = calibration factor.

Calibration factor = $D \times 50 / (E \times (F - G)$

where

D = mg component weighed,
E = mg internal standard,
F = relative peak area of component for the calibration solution, and
G = relative peak area of the same component for the blank solution.

B-2 METHOD II (DETERMINATION CARRIED OUT IN METHANOL)

B-2.1 Reagents

B-2.1.1 *Blank*, sample with very low solvent content.

B-2.1.2 *Internal Standard*, 3-methyl-2-pentanone.

B-2.1.3 *Methanol*

B-2.1.4 *Demineralized Water*

B-2.1.5 *Internal Standard Solution* — Add 50.0 ml methanol to a 50 ml injection vial and seal. Accurately weigh and inject (15 µl 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.

B-2.1.6 *Blank Solution* — Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of methanol and 1.0 ml of the internal standard solution. Heat at 60°C for 10 min and shake vigorously for 10 sec.

B-2.1.7 *Samples* — Weigh accurately 0.20 g sample into an injection vial. Add 5.0 ml methanol and add 1.0 ml of the internal standard solution. Heat at 60°C for 10 min and shake vigorously for 10 sec.

B-2.1.8 *Calibration Solution* — Solution A: Add 50.0 ml methanol to a 50 ml vial and seal. Accurately weigh, to within 0.01 mg, the vial and inject 50 µl of the component of interest through the septum. Reweigh the vial. Mix well.

Weigh into an injection vial, a known amount of blank (0.20 g), add 4.9 ml of methanol and 1.0 ml internal standard solution. Introduce 0.1 ml of Solution A into the injection vial. Mix well and heat at 60°C for 10 min and shake vigorously for 10 sec.

B-2.2 Procedure

Place the sample, blank and calibration samples in the sample tray of the head-space gas chromatograph — FID system. Analyse using the following analytical conditions.

B-2.2.1 *Column* — Fused silica, length 0.8 m, i.d. 0.53 mm, coated with DB-wax, film thickness 1 µm.

B-2.2.2 *Coupled with*, fused silica, length 30 m, i.d. 0.53 mm, coated with DB-1, film thickness 5 µm.

B-2.2.3

Conditions:

Carrier gas:	Helium
Flow rate:	208 kPa, 5 ml/min
Detector:	FID

Temperatures:	
Injector:	140°C
Oven conditions:	35°C for 5 min, then 5°C/min to 90°C, then 6 min at 90°C
Detector:	300°C
Head space sampler	
Sample heating temperature:	60°C
Sample heating period:	10 min
Syringe temperature:	70°C
Transfer temperature:	80°C
Sample gas injection:	1.0 ml in split mode

B-2.3 Calculation

$A \times B \times C/50$ = mg component per injection vial

where

A = relative peak area of the component concerned,
B = mg internal standard, and
C = calibration factor.

Calibration factor = $D \times 50 / (E \times (F - G) \times 10)$

where

D = mg component weighed,
E = mg internal standard,
F = relative peak area of component for the calibration solution, and
G = relative peak area of the same component for the blank solution.

(FAD 8)

Reprography Unit, BIS, New Delhi, India

Food Additives Sectional Committee, FAD 8

FOREWORD

This Indian Standard (Part 1) (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Divisional Council.

Chlorophyll, the green pigment of plants, is extracted and widely used as a colouring matter for various food items. It is obtained by solvent extraction of grass, lucerne, nettle and other plant material. During the subsequent removal of solvent, the naturally present co-ordinated magnesium may be wholly or partly removed from the chlorophylls to give the corresponding phaeophytins. The principal colouring matters are the phaeophytins and the magnesium chlorophylls and the extracted product from which the solvent has been removed contains other pigments such as carotenoids as well as oils, fats and waxes derived from source material.

This standard is one of a series of Indian Standards for natural food colours permitted under the *Prevention of Food Adulteration Rules*, 1955.

This standard was first issued in 1967 and covered the magnesium complex of chlorophyll only. It was later revised in 1986 into two parts, namely:

Part 1 Magensium complex
Part 2 Copper complex

This standard is being revised taking into consideration the latest publication for the food colour issued by JECFA and also the latest specifications laid down under the US FDA and the EEC Directives. In this revision requirement for the purity of chlorophyll and also the limit for residual solvent has been incorporated and requirements for heavy metal contaminants made more stringent to align with the international requirements.

Requirements for chlorophyll, food grade have been prescribed under the *Prevention of Food Adulteration Rules*, 1955 and due consideration has been given to the rules in the formulation of this standard. Due consideration has also been given to the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977. However, this standard is subject to restrictions imposed under these, wherever applicable.

Description

Common Name — Chlorophyll

Synonyms — Magnesium chlorophyll, magnesium phaeophytin, Cl Natural green 3, C.I. (1975) No. 75810; INS No. 140

C.A.S. Number — Phaeophytin *a*, Magnesium complex: 479-61-8

Phaeophytin *b*, Magnesium complex: 519-62-0

Chemical Names — The major colouring principles are:

Phytyl (13^2R, 17S, 18S) -3-(8-ethyl-13^2-methoxycarbonyl –2,7, 12,18-tetramethyl-13'-oxo-3-vinyl-13^1-13^2-17, 18 tetrahydrocyclopenta [at]-prophylrin-17-yl)propionate, (Phenophytin *a*), or as the magnesium complex (Chlorophyll *a*).

Phytyl (13^2R,17S, 18S)-3-(8-ethyl-7-formyl-13^2-methoxycarbonyl-2, 12, 18-trimethyl-13'-oxo-3-vinyl-13^1-13^2-17, 18-tetrahydro-cyclopenta [at]-prophylrin-17-yl)propionate, (Phenophytin *a*), or as the magnesium complex (Chlorophyll *a*).

Chemical Formula

Phaeophytin a magnesium complex (Chlorophyll *a*): $C_{55}H_{72}MgN_4O_5$

(Continued on third cover)

(*Continued from second cover*)

Phaeophytin *a*: $C_{55}H_{72}MgN_4O_5$

Phaeophytin *b*: Magnesium complex (chlorophyll *b*): $C_{55}H_{70}MgN_4O_6$

Phaeophytin *b*: $C_{55}H_{72}N_4O_6$

Molecular Weight

Phaeophytin *a*: magnesium complex (Chlorophyll *b*) 893.51

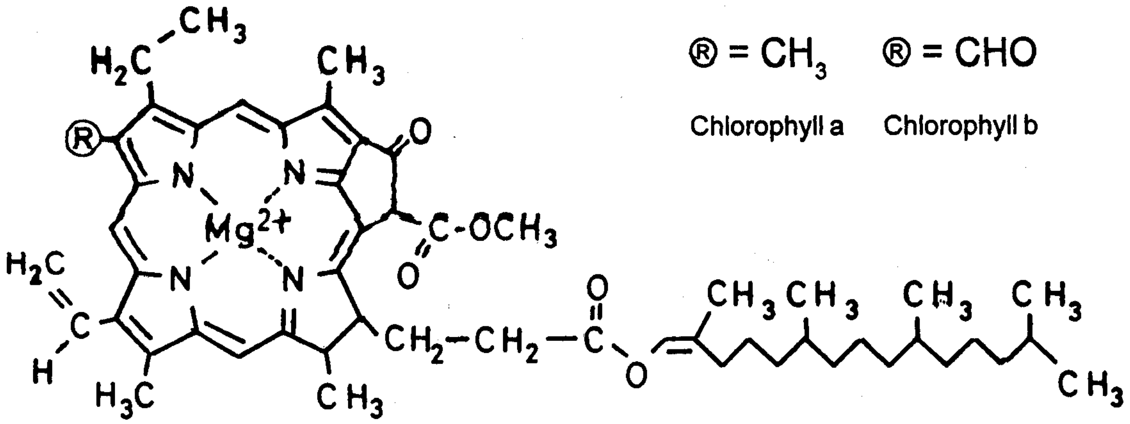
Phaeophytin *a*: 871.22

Phaeophytin *b*: magnesium complex (Chlorophyll *b*) 907.49

Phaeophytin *b*: 885.20

Solubility — Insoluble in water; soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and fixed oils.

The structural formula of chlorophyll is given below:



Chlorophyll Magnesium Complex

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 :1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

CHLOROPHYLL, FOOD GRADE — SPECIFICATION

PART 1 MAGNESIUM COMPLEX

(Second Revision)

1 SCOPE

This standard (Part 1) prescribes requirements and methods of sampling and test for chlorophyll, food grade.

2 REFERENCES

The following standards contain provisions, which through reference in this text constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1994	Methods of sampling and test for food colours (*second revision*)
2491 : 1998	Food hygiene — General principles — Code of practice (*second revision*)

3 DESCRIPTION

Chlorophyll is a waxy solid ranging in colour from olive green to dark green depending on the content of coordinated magnesium.

4 REQUIREMENTS

4.1 Thin-Layer Chromatography

Apply a 1 in 20 solution of the sample in chloroform as a band of the length of 2 cm to a Silica 60 TLC plate. After drying, develop the plate by a mixture of 50 percent hexane, 45 percent chloroform and 5 percent ethanol (General purpose reagent grade chloroform is supplied with 2 percent of added ethanol as a stabilizer. The 5 percent ethanol in the solvent mixture is in addition to this), until the solvent ascends to a point 15 cm above the initial spots. Allow the solvent to evaporate, then visually examine the separated spots and identify the components of interests by their R_f values and colours.

Approximate R_f values and colour of the spots are as follows:

a) Phaeophytin *a*: 0.77, grey/brown;
b) Phaeophytin *b*: 0.75, yellow/brown;
c) Chlorophyll *a*: 0.50, blue/green; and
d) Chlorophyll *b*: 0.63, yellow/green.

In addition spots may be visible for β-carotene at R_f 0.81 and xanthophyll at R_f 0.47 and 0.23.

4.2 The material shall also conform to the requirements given in Table 1.

4.3 The product shall be processed, packed, stored and distributed under hygienic conditions in licenced premises (*see* IS 2491).

Table 1 Requirements for Chlorophyll
(*Clause* 4.2)

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Annex of This Standard	Clause of IS 169
(1)	(2)	(3)	(4)	(5)
i)	Total combined phaeophytins and their magnesium complexes, percent by weight, *Max*	10	A	—
ii)	Residual solvents, mg/kg, *Max* Acetone, methanol, ethanol, propan-2-ol, hexane[1)]	50	—	—
iii)	Dichloromethane[1)]	10	—	—
iv)	Arsenic (as As), mg/kg, *Max*	3	—	15
v)	Lead (as Pb), mg/kg, *Max*	10	—	15

[1)] Method of test under preparation.

5 PACKING, STORAGE AND MARKING

5.1 Packing

The material shall be filled in amber-coloured glass containers or any other suitable containers under an inert gas as carotene is sensitive to oxygen and light. The containers shall be such as to preclude contamination of the contents with metals or other impurities.

5.2 Storage

The material shall be stored in a cool and dry place so as to avoid excessive exposure to heat.

5.3 Marking

Each container shall be legibly and indelibly marked with the following information:

a) Name of the material including the words 'Food Grade';
b) Name and address of the manufacturer;
c) Batch or code number;
d) Net content when packed;
e) Instruction for storage;
f) Best before .... (Month and year to be given by the manufacturer); and
g) Any other requirements as given under the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977 and *Prevention of Food Adulteration Act*, 1955 and the Rules.

5.3.1 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

5.3.1.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6 SAMPLING

Representative samples of the material shall be drawn according to the method prescribed in **4** of IS 1699.

7 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

ANNEX A

[*Table* 1, *Sl No.* (i)]

METHOD FOR ASSAYING PURITY OF CHLOROPHYLL

A-0 METHOD

The assaying of purity of chlorophyll is carried out by the Spectrophotometric method.

A-1 REAGENT

A-1.1 Diethyl Ether, spectrophotometric grade.

A-1.2 Oxalic Acid

A-2 APPARATUS

A-2.1 Spectrophotometer, capable of accurate (± 1 percent or better) measurement of absorbance in the region of 350-750 nm with an effective slit width of 10 nm or less.

A-2.2 Absorption Cells, 1 cm light path.

A-3 PROCEDURE

Accurately weigh about 100 mg of the sample and dissolve in diethyl ether, making the volume to 100 ml. Dilute 2 ml of this solution to 25 ml with diethyl ether. The concentration of the sample should not give an absorbance at 660.4 nm that is in excess of the working range for absorbance measurements, that is, not in excess of 0.7. Measure the absorbances of the solution in a 1-cm cell against a diethyl ether blank at 660.4 nm, 642.0 nm, 667.2 nm, and 654.4 nm (These being the absorbance maxima in diethyl ether for chlorophyll *a*, chlorophyll *b*, phaeophytin *a*, and phaeophytin *b*, respectively). In addition measure at 649.8 nm and 628.2 nm. To the remaining diluted solution add one crystal of oxalic acid and after dissolution and mixing, remeasure the absorbances at the same wavelengths. 'ΔA' is the difference between the absorbances between the absorbance at the respective wavelengths, before and after addition of oxalic acid.

Calculate the concentration of the individual compounds in micromoles per litre from the following equation:

Chlorophyll a = $17.7\ \Delta A\ (660.4\ \text{nm}) + 7.15\ \Delta A\ (642.0\ \text{nm})$

Chlorophyll b = $19.4\ \Delta A\ (642.0\ \text{nm}) - 2.92\ \Delta A\ (660.4\ \text{nm})$

Phaeophytin a = $4.89\ A\ (649.8\ \text{nm}) + 0.054\,9\ A\ (628.2\ \text{nm}) + 18.7 A\ (667.2\ \text{nm}) + 0.057\,5\ A\ (654.4\ \text{nm}) - \text{chlorophyll}\ a$

Phaeophytin b = 71.0A (649.8 nm) + 2.51 A (628.2 nm) −13.5 A (667.2 nm) + 84.3 A (654.4 nm) — chlorophyll b

Convert the figures inmicromoles/litre to percentages using the following equations:

$$\text{Percent chlorophyll, } a = \frac{\text{Micromoles} \times 0.893\,5 \times 12.5 \times 100}{\text{Mass of sample, mg}}$$

$$\text{Percent chlorophyll, } b = \frac{\text{Micromoles} \times 0.907\,5 \times 12.5 \times 100}{\text{Mass of sample, mg}}$$

$$\text{Percent phaeophytin, } a = \frac{\text{Micromoles} \times 0.871\,2 \times 12.5 \times 100}{\text{Mass of sample, mg}}$$

$$\text{Percent phaeophytin, } b = \frac{\text{Micromoles} \times 0.885\,2 \times 12.5 \times 100}{\text{Mass of sample, mg}}$$

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act,* 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Copyright

BIS has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of BIS. This does not preclude the free use, in the course of implementing the standard, of necessary details, such as symbols and sizes, type or grade designations. Enquiries relating to copyright be addressed to the Director (Publications), BIS.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

This Indian Standard has been developed from Doc : No. FAD 8 (1471).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters :

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110 002
Telephones : 2323 0131, 2323 3375, 2323 9402

Telegrams : Manaksanstha
(Common to all offices)

Regional Offices :		Telephone
Central	: Manak Bhavan, 9 Bahadur Shah Zafar Marg NEW DELHI 110 002	2323 7617 2323 3841
Eastern	: 1/14 C.I.T. Scheme VII M, V. I. P. Road, Kankurgachi KOLKATA 700 054	2337 8499, 2337 8561 2337 8626, 2337 9120
Northern	: SCO 335-336, Sector 34-A, CHANDIGARH 160 022	260 3843 260 9285
Southern	: C.I.T. Campus, IV Cross Road, CHENNAI 600 113	2254 1216, 2254 1442 2254 2519, 2254 2315
Western	: Manakalaya, E9 MIDC, Marol, Andheri (East) MUMBAI 400 093	2832 9295, 2832 7858 2832 7891, 2832 7892

Branches : AHMEDABAD. BANGALORE. BHOPAL. BHUBANESHWAR. COIMBATORE. FARIDABAD. GHAZIABAD. GUWAHATI. HYDERABAD. JAIPUR. KANPUR. LUCKNOW. NAGPUR. PARWANOO. PATNA. PUNE. RAJKOT. THIRUVANANTHAPURAM. VISAKHAPATNAM.

Printed by Sunshine Graphics